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Colorado 80403 (US). **GHIRARDI, Maria, L** [US/US]; 111354 20th Avenue, Lakewood, Colorado 80215 (US). **SEIBERT, Michael** [US/US]; 13134 Yale Place, Lakewood, Colorado 80228 (US).

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(74) **Agents:** **WHITE, Paul, J.** et al.; National Renewable Energy Laboratory, 1617 Cole Boulevard, Golden, Colorado 80401 (US).

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(71) **Applicant (for all designated States except US): MIDWEST RESEARCH INSTITUTE [US/US]; 425 Volker Boulevard, Kansas City, Missouri 64110 (US).**

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(72) Inventors: and

(75) **Inventors/Applicants (for US only):** KING, Paul
[US/US]; 1919 Denver West Drive, Apt. 122B, Golden,

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(54) Title: OXYGEN-RESISTANT HYDROGENASES AND METHODS FOR DESIGNING AND MAKING SAME

CaI	PVA	AEK	EH	ERK	SG	HAL	ND	P	EH	V	Y	A	N	S	G	T	A	G	L	E	K	M	G	V	K	D	247			
CpI	PVA	AE	SE	X	S	H	DR	K	X	N	A	L	N	A	P	E	H	V	A	M	S	G	L	E	K	M	G	V	248	
Dd	PEN	E	Y	S	A	Q	S	W	P	E	E	K	L	K	D	G	V	R	C	A	M	P	E	A	Y	A	G	98		
CrHydA2	ATD	P	SH	W	K	L	A	E	E	D	R	P	K	D	G	G	N	V	C	Q	Q	V	E	A	Y	A	S	110		
CrHydA1	AAEP	T	PH	V	Q	Q	L	A	E	E	A	K	P	K	D	D	P	E	H	C	Q	Q	V	E	A	Y	A	113		
CaI	VT	AE	E	Y	T	A	E	M	K	E	E	K	E	P	I	N	G	H	E	V	E	A	N	S	G	L	E	K	289	
CpI	VT	AE	E	Y	T	A	E	M	K	E	E	K	E	P	I	N	G	H	E	V	E	A	N	S	G	L	E	K	290	
Dd	VT	E	K	L	A	A	Q	E	K	E	A	R	C	W	E	T	E	D	V	E	N	W	G	E	E	T	K	142		
CrHydA2	SP	E	K	A	T	C	A	E	A	T	E	E	T	E	L	A	L	E	L	E	N	W	G	E	E	T	K	163		
CrHydA1	TP	E	K	A	T	C	A	E	R	E	E	T	E	L	A	L	E	L	E	N	W	G	E	E	T	K	160			
CaI	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	339	
CpI	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	340	
Dd	N	E	A	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	342
CrHydA2	E	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	213	
CrHydA1	E	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	210	
CaI	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	386	
CpI	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	387	
Dd	E	Y	D	E	Q	V	T	V	S	H	V	C	A	A	K	E	G	L	E	P	L	E	S	S	G	---	E	D	239	
CrHydA2	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	262	
CrHydA1	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	260	
CaI	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	436	
CpI	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	437	
Dd	E	K	A	O	E	K	A	K	P	G	K	K	S	L	E	S	E	S	G	G	A	T	E	G	E	V	E	289		
CrHydA2	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	312	
CrHydA1	G	E	F	E	E	V	S	G	S	G	E	R	L	Q	N	T	H	E	D	R	F	E	A	S	E	R	I	P	310	
CaI	X	E																												

(57) Abstract: The invention provides oxygen-resistant iron-hydrogenases ([Fe]-hydrogenases) for use in the production of H₂. Methods used in the design and engineering of the oxygen-resistant [Fe]-hydrogenases are disclosed, as are the methods of transforming and culturing appropriate host cells with the oxygen-resistant [Fe]-hydrogenases. Finally, the invention provides methods for utilizing the transformed, oxygen insensitive, host cells in the bulk production of H₂ in a light catalyzed reaction having water as the reactant.

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